HORMONAL CONTROL OF RESPIRATORY METABOLISM DURING GROWTH, REPRODUCTION, AND DIAPAUSE IN MALE ADULTS OF PYRRHOCORIS APTERUS L. (HEMIPTERA) [1]

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In many species of insects the larval stages do not accumulate sufficient reserve materials to provide for egg maturation in the adult female. Such females may consume a considerable amount of food after emergence as adults and their body size and metabolic activity increase. In most of these cases nutrition and reproduction are under hormonal control. The corpus allatum hormone (CAH) activates the ovaries and the accessory glands of the reproductive tract; in the absence of the hormone, yolk deposition cannot proceed and the ovaries and accessory glands remain inactive (reviews by Wigglesworth, 1954; Pflugfelder, 1958; Novák, 1960). Furthermore, the activation hormone (AH) from the cerebral neurosecretory cells-corpora cardiaca may also be necessary for the deposition of yolk, apparently through its control over enzyme secretion in the alimentary tract (Thomsen and Møller, 1963).

In contrast to the females, however, growth and reproduction of the adult male appears to depend little, if at all, on hormones, even in those species in which hormones control the reproductive activities of the female. Thus, in most cases the process of spermatogenesis appears to be independent of hormones although the corpus allatum hormone may in some cases stimulate the growth and function of the accessory reproductive glands (Wigglesworth, 1936; Thomsen, 1942; Scharrer, 1946; Scharrer and von Harnack, 1958; Pflugfelder, 1958; Johansson, 1958). Janda and Sláma (1964) have shown that much less reserve material is metabolized during spermatogenesis than during egg maturation. The post-emergence somatic growth is therefore usually less in males than in females, as is the total metabolic activity. Similarly, the difference between active and diapause development is less apparent in males than it is in females (Ushatinskaya, 1957; Hodek and Čerkasov, 1963).

In an earlier paper Sláma (1964) showed that the respiratory rate in females of *Pyrrhocoris* is cyclical, increasing and decreasing periodically in close connection with the cycles of reproduction and oviposition. The cycles disappear after the corpus allatum or the ovaries are removed; the females then maintain a constant

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intermediate rate of respiration. When the centers engaged in the release of the activation hormone (*i.e.*, the neurosecretory cells of the brain or the corpora cardiaca) are also removed, the postemergence growth of the females is suppressed and the respiration rate decreases to the level characteristic of diapause.

Sláma (1964) has therefore recognized three components in the respiratory metabolism of adult *Pyrrhocoris* females, each component having a characteristic response to hormones. The three components are: (a) The reproductive metabolism, which depends on the CAH or on the presence of ovaries; (b) digestive metabolism, which is independent of the CAH but depends on AH; and (c) basic metabolism of cells and muscle metabolism, which are not dependent on hormones and are identical with diapause metabolism. However, both the hormones may affect basal metabolism indirectly by activating specific tissues. Thus, the overall metabolism of an active female is controlled by the simultaneous cooperation of hormones; the CAH regulates metabolism in tissues associated with reproductive functions, whereas the AH regulates metabolism in tissues associated with trophic activity.

In the present study the relations between hormones and respiration have been investigated in adult males of *Pyrrhocoris*. The results are compared with findings obtained on female adults (see Sláma, 1964).

Materials and Methods

Adult males of *Pyrrhocoris apterus* L. were used in all experiments. They were kept individually without females at 25° C. The techniques for rearing *Pyrrhocoris*, for operating on them, and for determining their oxygen consumption were described earlier (Sláma, 1964). The respiration rate of each specimen was measured at daily intervals at 25° C. The respiration data for specimens in which the operations were unsuccessful, or which died during the experiment were omitted from the final averages. At the end of each experiment the males were dissected and anatomical changes were noted.

Results

1. *Growth and oxygen consumption of the normal male* (Fig. 1)

The process of spermatogenesis commences before the end of the last larval stage, and as early as two or three days after adult emergence the accessory glands have increased in size, the testes and the seminal vesicles contain spermatozoa and the male will mate. The increase in body weight is relatively small, and is restricted to the first three days of adult life; thereafter it remains constant at a level of about 55 or 60 mg. In females the body weight increases up to 80 mg. during the first 3 to 6 days of adult life, although the initial weight is about 40 to 55 mg. in both sexes. The considerable loss of weight which follows each oviposition in females is quickly compensated by a new intake of food.

The oxygen consumption was measured in a total of 20 males, three of which had pathologically swollen abdomens and were discarded. The remainder were used for Figure 1 b. For comparison, growth and oxygen consumption curves for normal females are given in Figure 1 a (from Sláma, 1964). A further 32 males were used to confirm the values for oxygen consumption at 15 days.

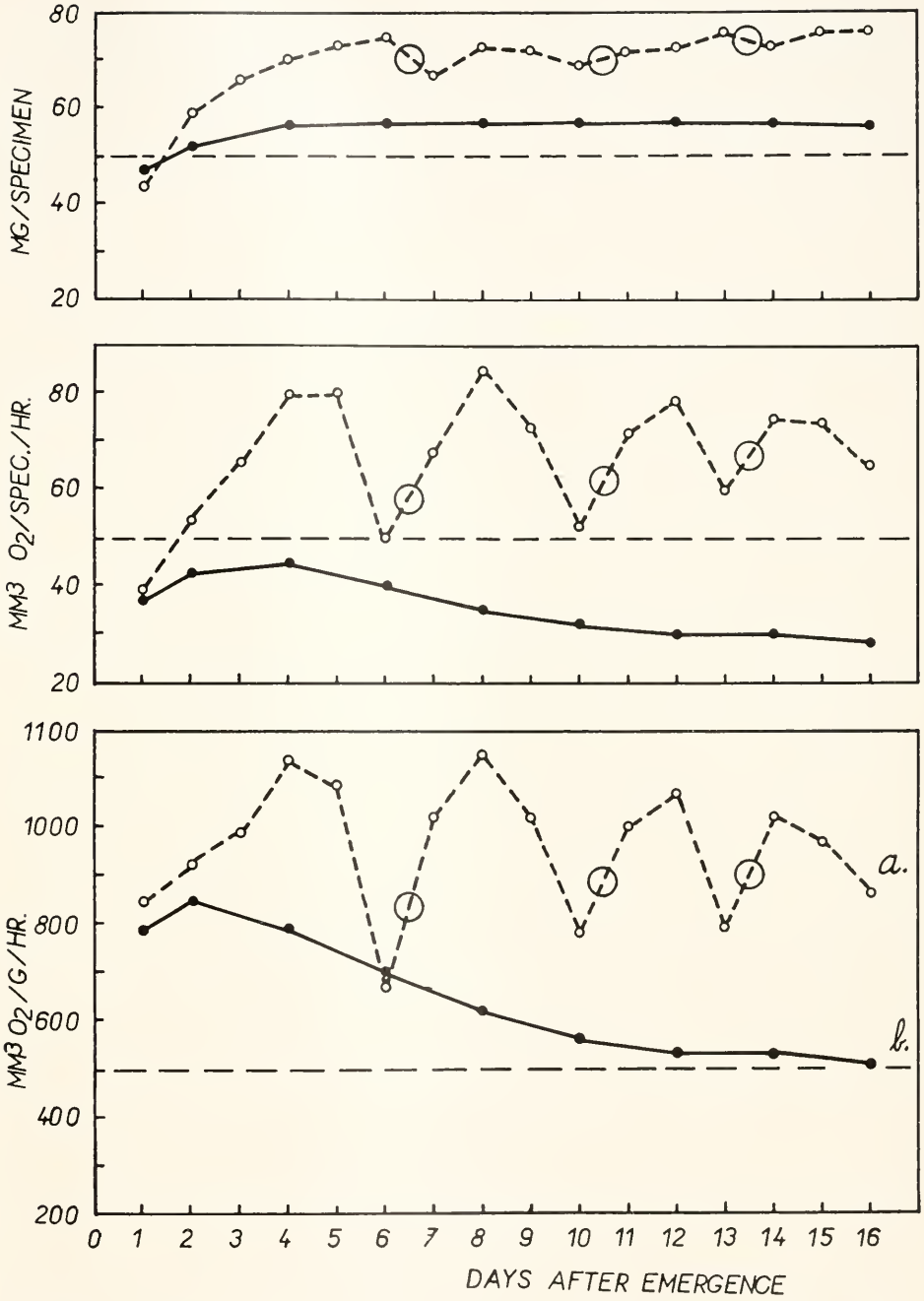

FIGURE 1. Changes in body weight (above), oxygen consumption per specimen (middle), and oxygen consumption per gram of the live weight (below) during adult life of normal females (a) (after Sláma, 1964), and of normal males of *Pyrrhocoris apterus* L. (b). Open rings indicate the moment of oviposition.

The oxygen consumption of the normal male appears to increase slowly during the growth period of the first three days; after this it diminishes steadily to stable values of about 400 to 500 mm.³/gm./hr., where it remains (Fig. 1 b). There are no cycles of oxygen consumption in the normal males which would correspond to the cycles found in females. Furthermore, the rate of respiration in the normal male is very low when compared with that of the normal female; the maximal respiration rate in the female may be two or three times the stable rate of the male.

2. Operated controls (Fig. 2)

As a control experiment for the allatectomies and cardiac-allatectomies described below, ten males were subjected to narcosis and the operation through the neck membrane was performed, except that the glands were not removed (see Sláma, 1964). One of the operated animals died, and the remaining nine were used for Figure 2 c.

As Figure 2 c shows, the body weight and oxygen consumption of the operated males resemble those of the normal males. Thus, neither narcosis nor the neck membrane incision, *per se*, had an effect on growth and respiration.

3. Allatectomized males (Fig. 2 d)

Of 22 operated males, three died, and three had the corpus allatum incompletely removed; the remaining 16 were used for Figure 2 d. A further 25 allatectomized males were used to check the values of oxygen consumption at 15 days.

Anatomically, the allatectomized males differ little from the normal males. Their testes and seminal vesicles contain spermatozoa and they are able to mate. However, the accessory reproductive glands are subnormal in size in at least half of the allatectomized males.

The changes in the body weight of allatectomized males are similar to those of normal males or operated controls. The failure of the body weight to increase after allatectomy indicates that reserve material does not accumulate as it does in allatectomized females. During an initial period of about six days after emergence and operation, the oxygen consumption of allatectomized males appears slightly lower than that of normal males or controls. However, the later stable value for the respiration rate is similar to that in the other groups (Fig. 2 d). The oxygen consumption of the 25 additional allatectomized males was consistent with the figures given in Figure 2 d, the average being 470 mm.³ O_2/gm./hr.

From these results it is evident that the removal of the corpus allatum has little effect on the respiratory metabolism of *Pyrrhocoris* males. This contrasts with the situation in females, where allatectomy considerably depresses the rate of respiration.

4. Cardiac-allatectomized males

Of 15 cardiac-allatectomized males, three died, one had the corpora cardiaca incompletely removed and 11 survived. The weight and oxygen consumption were measured only at the first, fourth, twelfth and seventeenth days after emer-

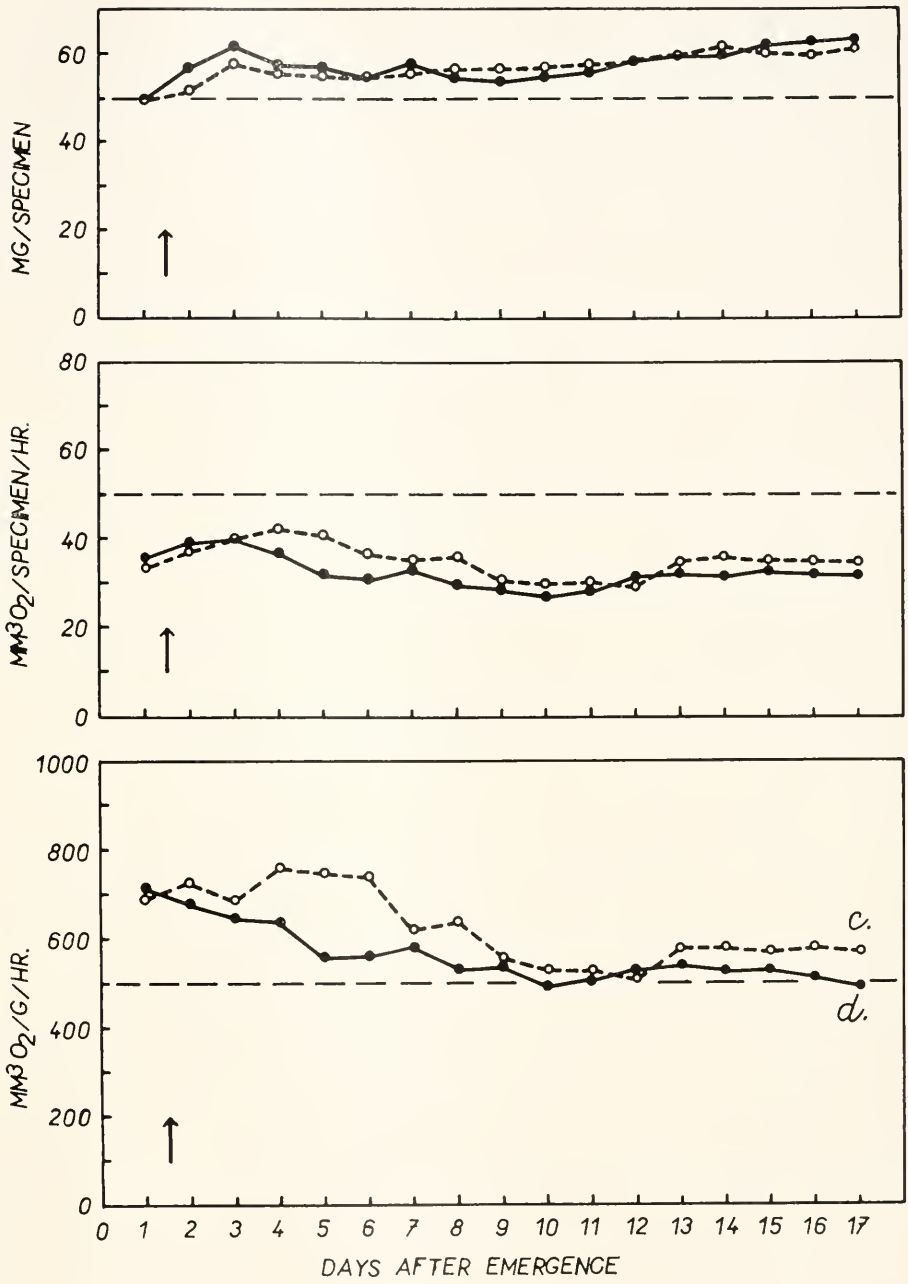

FIGURE 2. Changes in body weight (above), oxygen consumption per specimen (middle), and oxygen consumption per gram of live weight (below) during adult life of sham-operated (c) and allatectomized (d) males of *Pyrrhocoris*. Arrows indicate the moment of operation.

gence and operation. The rate of respiration at 15 days was measured on an additional series of 18 cardiac-allatectomized males.

The changes in the rates of growth and oxygen consumption during adult life in cardiac-allatectomized males were again similar to those of normal or allatectomized males. The additional values at 15 days, averaging 452 $mm.^3/gm./hr.$, confirm that the stable rate of respiration in cardiac-allatectomized males does not differ from that in the other experimental groups.

The results show that not only allatectomy but also cardiac-allatectomy has little if any effect on the respiration rate of *Pyrrhocoris* males. This again contrasts sharply with the situation in females, where cardiac-allatectomy reduces the respiration rate to as little as one third of the normal level. However, the rate of respiration in cardiac-allatectomized or normal males is very similar to that of cardiac-allatectomized females.

5. *Castrated males* (Fig. 3)

Of 15 males that emerged from larvae castrated in the last instar, two died and two were incompletely castrated. The remaining 11 were used for Figure 3 e. Additional measurements at 15 days were taken on a series of 17 castrated males.

The curves for growth and respiration of the castrated males are again similar to those of the other males (Fig. 3 e), as are the values at 15 days. These results show that the respiratory metabolism of testicular tissue is insignificant relative to the metabolism of the rest of the animal. In the adult female, on the other hand, the removal of the ovaries results in a depression of the respiration rate.

6. *Diapausing males* (Fig. 3)

To induce diapause, males were kept under a short photoperiod (8 hours of light per day) from the beginning of the 5th instar; the experimental conditions were otherwise unaltered. Measurements of 11 diapausing males with undeveloped accessory glands were used for Figure 3 f. Specimens in which these glands had been found developed normally after dissections were omitted.

Morphologically, the diapausing and active males are closely similar but the accessory glands of the former are usually less developed and the potential for mating is limited. The growth and respiration curves of diapausing males again follow the course common to the other experimental series. In diapausing females, on the other hand, respiration is greatly suppressed, reaching levels that are very similar to the respiration rates of the males. Thus, as far as respiration is concerned one may consider all the males (whether normal, allatectomized, cardiac-allatectomized or castrated) to be in "diapause" when compared with females.

7. *The effect of mating on respiration in the male* (Fig. 4)

The data in the preceding sections have shown that the respiratory metabolism of the males, unlike that of the females, is largely independent of hormones or gonads. Because none of the males was allowed to mate, it could be argued that

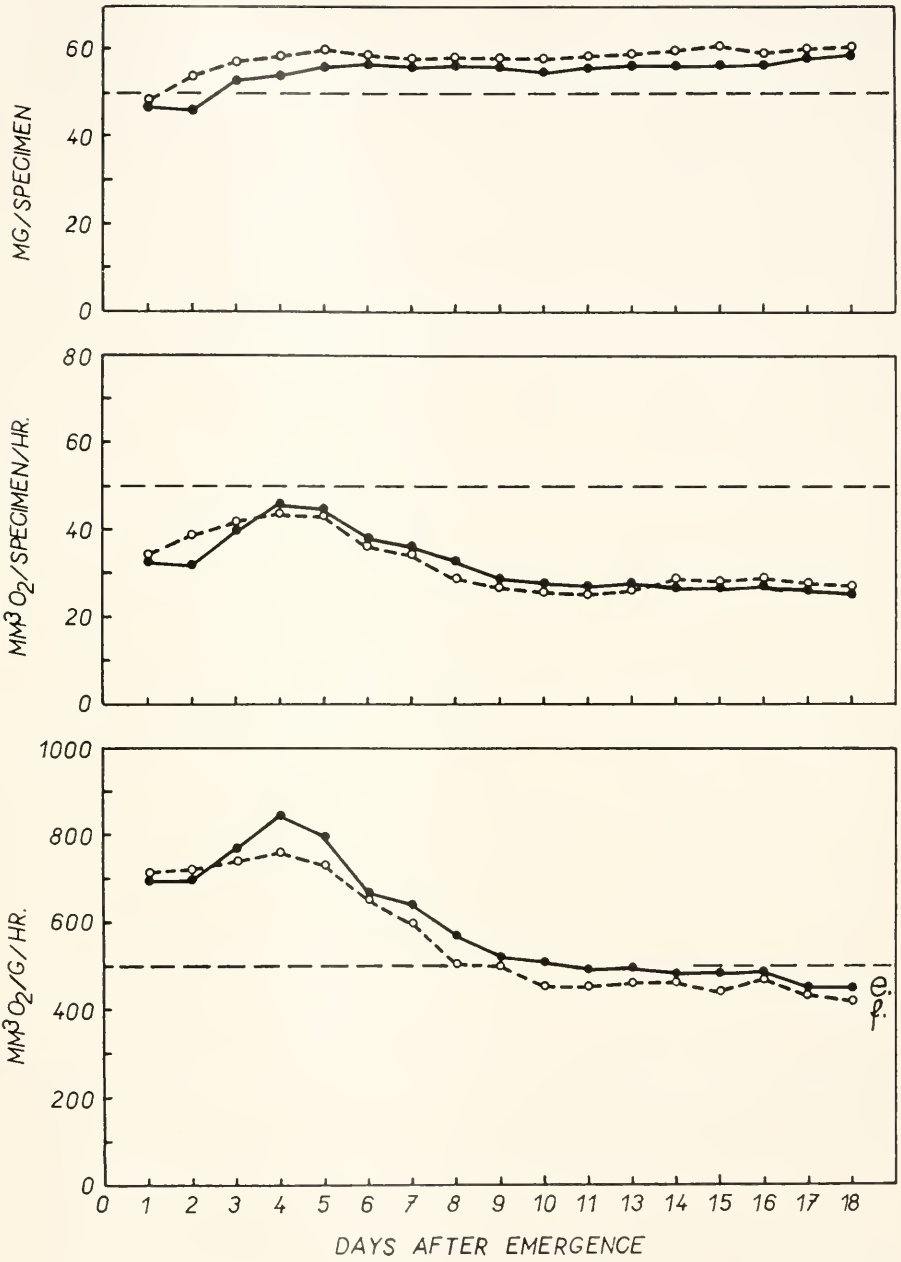

FIGURE 3. Changes in body weight (above), oxygen consumption per specimen (middle), and oxygen consumption per gram of live weight (below) during adult life of castrated (e), and diapausing (f) males of *Pyrrhocoris*.

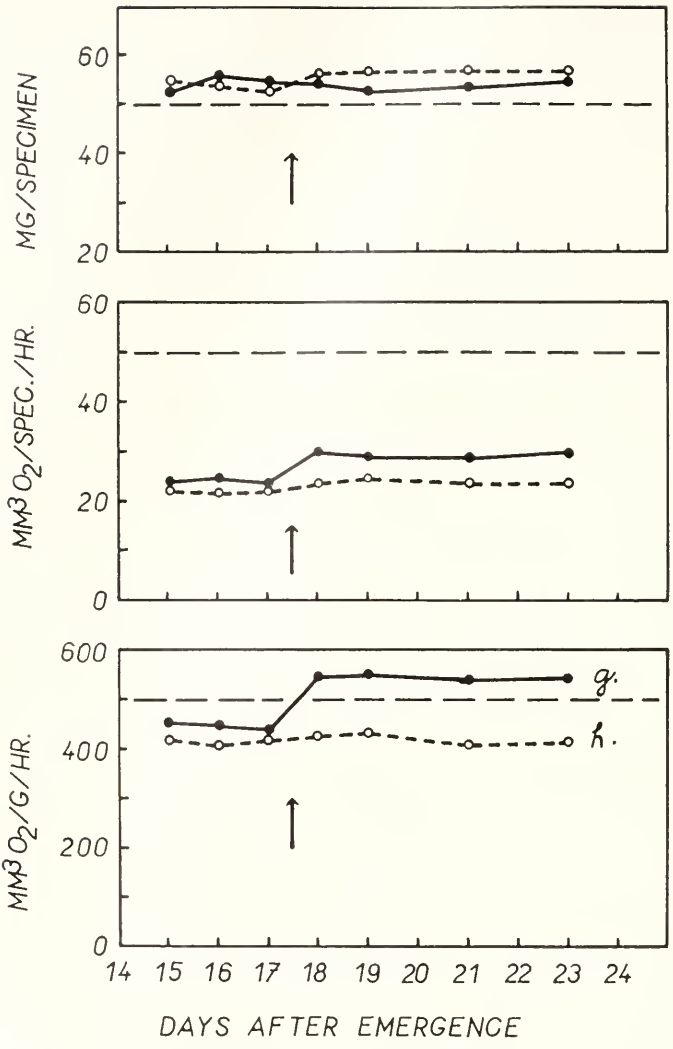

Figure 4. Changes in body weight (above), oxygen consumption per specimen (middle), and oxygen consumption per gram of live weight (below) of normal males (g), and allatectomized (h) males of *Pyrrhocoris* which were allowed to mate. Arrows indicate the beginning of mating activity.

the apparent independence is a consequence of sexual inactivity. To clarify this point, the rate of respiration was measured in 11 intact males, aged 15 days, first without females, and then after the males had mated with up to three females. After such intensive mating the rate of respiration increases about 20% (Fig. 4 g). It is possible that the increase is connected with the synthesis of material in the depleted accessory glands. Similar experiments therefore were carried out on 11 allatectomized males also aged 15 days. The mating activity in this case was not

followed by increase in the rate of respiration (Fig. 4 h). Furthermore, the size of the accessory glands of allatectomized males increases very little after mating. These results support the assumption that the effect of intensive mating on the respiration of the normal males is connected with changes in the synthetic activity of the accessory glands.

8. *The effect of feeding on the respiration of males*

Adult males of *Pyrrhocoris* consume relatively little food when compared with females. As the digestion and utilization of food influence the respiration of adult females, it was necessary to determine the extent to which the respiration of males depends on these factors. Eleven males were therefore starved for three months in a refrigerator at 10° C., and their rates of respiration were measured. They were then transferred to 25° C., their respiration was remeasured, and they were then fed. The rate of respiration in these males increased after feeding; the oxygen consumption at 25° C. rose from approximately 330 mm.^{3}/gm./hr. to 500 mm.^{3}/gm./hr. after feeding. Thus, processes associated with feeding influence the respiratory metabolism of males, although the effect is minor when compared with that found in females (see Sláma, 1960). The results also show that prolonged starvation decreases the respiratory rate of males, as it does in females; presumably, the effect depends on the reduction of metabolic processes that are normally independent of hormones.

9. *The effect of implanting female corpora allata on the respiration of males*

The independence of respiration on hormones in male *Pyrrhocoris* might be due to one of two factors: (a) The corpus allatum may be inactive. Certainly the corpora allata of male *Pyrrhocoris* are much smaller than those of the female in which the glands secrete actively. (b) The presumptive target organ (*e.g.*, the reproductive system) may not respond to the CAH.

To test these possibilities, each of 10 male *Pyrrhocoris*, 15 days old, was implanted with a corpus cardiacum-corpus allatum complex from a single active female; such an implantation into diapausing or cardiac-allatectomized females causes a rapid increase in oxygen consumption and in body weight (Sláma and Hrubešová, 1963). The oxygen consumption was measured before the operation and on the third and fifth days thereafter. Eight specimens survived. The results showed that the operation was followed neither by increased oxygen consumption nor by an increase in body weight, confirming that the hormones released from the corpora allata or the corpora cardica act only on specific tissues; effects on general metabolism, as in the females, are indirect. Thus, even a high concentration of hormone may be inactive metabolically if there are no tissues which are able to respond.

Discussion

In females of various insect species, allatectomy leads to a decrease in oxygen consumption, whereas implantation of the active corpus allatum causes an increase (see Sláma, 1964). On the other hand, the present results show that the respiratory metabolism of males is largely or completely independent of hormonal activity.

Similar results have been obtained in *Calliphora*, where the implantation of extra corpora allata has no effect on respiration in the male, although it increases oxygen consumption in the female. Allatectomy also has less effect on the respiration of male *Calliphora* than it does on that of the female (Thomsen, 1949). Furthermore, the cytochrome oxidase activity in male *Drosophila* is independent of hormones (Bodenstein and Sacktor, 1952).

The respiration rate of male *Pyrrhocoris* is only a third to a half that of the active female (see Sláma, 1964). Two of the three components of respiratory metabolism which occur in females have no counterpart in males, *i.e.*, the active reproductive metabolism and digestive metabolism. One may therefore conclude that the respiratory metabolism of males is equivalent to the third type of female metabolism, *i.e.*, the basic metabolism of cells and muscles, or the metabolism of diapause. This component has a common characteristic in both sexes; it is independent of hormones and it is most evident when growth, digestion and reproduction are depressed to a minimum. This type of respiration is characterized by an oxygen consumption of about 20 to 25 $mm.^3$/hr./specimen in normal males and in females from which the corpora cardiaca and corpora allata had been removed (Sláma, 1964).

In the females the CAH regulates reproductive metabolism, probably by direct control over the ovarian tissue. The AH, on the other hand, regulates digestive metabolism, which is also probably a direct effect on the alimentary tract. This may explain the similarity between the respiratory metabolism of the normal male and the low metabolism of the females deprived of hormonal sources. The metabolic activity associated with reproduction and digestion is reduced so greatly in the normal male that any action of the hormones on overall metabolism is correspondingly reduced. In females Janda and Sláma (1964) have shown that when the activation hormone and digestion are working in the absence of the CAH or ovarian growth (as in allatectomized females), digestive activity leads to an enormous accumulation of reserve products. This is, however, not the case in males, where the metabolism of the intestinal tissue is little stimulated by the AH. These facts indicate that the total capability and the sensitivities of appropriate tissues are critical factors to be considered in the overall physiological interpretation of hormone action.

The critical difference between male and female metabolism in *Pyrrhocoris* therefore depends on the difference in the demands of the reproductive systems; the metabolism involved in basal maintenance is probably similar in the two sexes. In the female the loss of materials with the eggs, which may involve up to a third of the body weight, must be compensated by additional ingestion and digestion. As Sláma and Hrubešová (1963) have shown, the total amount of oxygen consumed for the production of all maturing eggs is very large. In the male, on the other hand, production of the spermatozoids involves the use of little reserve material, and in consequence castration has little effect on respiration. There is not much change in the size of the testes during maturation, and the major metabolic outlay arises most probably from the secretory activity of the accessory glands.

It is with the latter activity that the "reproductive metabolism" of the male is concerned, and this is a common phenomenon in insects. The CAH stimulates secretory activity in the accessory glands of *Rhodnius* (Wigglesworth, 1936), in

Calliphora (Thomsen, 1942), in *Schistocerca* (Loher, 1960), etc. Clearly, though, the glands in *Pyrrhocoris* are too small for hormonally induced changes in their activity to influence respiratory activity as a whole; it is only after gross depletion that such changes can affect respiration.

At present it is not possible to extrapolate from the results obtained on *Pyrrhocoris* to males of other insects. Stable and low levels of respiration have been found in adult males of *Leucophaea* in which the females have cyclical respiratory activity which may surpass that of the male (Sägesser, 1960). Yet in some species the respiration rate of males is higher than that of females, as in *Locusta* (Butler and Innes, 1936). In *Calliphora* (Thomsen, 1949) and in *Leptinotarsa* (Ushatinskaya, 1957) the respiration of both sexes is approximately equal. These differences cannot be evaluated without considering the processes which may contribute to them, such as the specific differences in the activity of the accessory glands, reproductive system, locomotor activity and the amount of inert reserve material.

The adult life of *Pyrrhocoris* males may last from two months to one year, depending mostly on their sexual activity. Except the brief time interval after adult ecdysis, the males keep a constant body size, reduced food ingestion, and low and steady respiration rate with uniform locomotory activity. This makes the male resistant against unfavored conditions of life, starvation, etc. Evidently, the main biological function of these males depends merely in their preservation for the purpose of female insemination. The induction of diapause has, therefore, little, if any effect on growth, respiration rate, locomotory activity or food ingestion rate, the differences between the normal and the diapausing males being restricted to the changes that occur in the accessory sexual glands. These observations may also demonstrate the lack of the hormonal stimulation of metabolism in the males of *Pyrrhocoris*, because diapause in insects is generally considered as a result of hormonal deficiency. In females of *Pyrrhocoris*, where the hormonal regulation of metabolic processes appears to be apparent, the induction of diapause is always followed by a suppression of growth and metabolism (Sláma, 1964).

Summary

The respiratory metabolism of adult *Pyrrhocoris* males is very low when compared with that of females, and it is independent of hormones. Allatectomy, cardiac-allatectomy, castration and diapause have no profound effect on the rate of respiration in the adult male, unlike the situation in the adult female. Indeed, males respire at about the same rate as females deprived of endocrine glands. The sexual dimorphism in respiration and hormone action is correlated with the differences in growth, digestion and reproduction. It is shown that the hormones influence the over-all metabolism indirectly by regulating the morphology and physiological activity of specific tissues.

Literature Cited

Bodenstein, D., and B. Sacktor, 1952. Cytochrome c oxidase activity during the metamorphosis of *Drosophila virilis*. *Science*, **116**: 299–300.

Butler, C. G., and J. M. Innes, 1936. A comparison of the rate of metabolic activity in the solitary and migratory phases of *Locusta migratoria*. *Proc. Roy. Soc. London, Ser. B*, **119**: 296–304.

HODEK, I., AND J. ČERKASOV, 1963. Imaginal dormancy in *Semiadalia undecimnotata* Schneid. II. Changes in water, fat and glycogen content. *Acta Soc. Zool. Bohemoslov.*, **27**: 298–318.

JANDA, V., JR., AND K. SLÁMA, 1964. Einfluss von Hormonen auf den Glykogen-, Fett-, und Stickstoffmetabolismus bei Adulten *Pyrrhocoris apterus* L. (Hemiptera). *Zool. Jahrb. Physiol.*, **70** (in press).

JOHANSSON, A. S., 1958. Relation of nutrition to endocrine-reproductive functions in the milkweed bug *Oncopeltus fasciatus* (Dallas) (Heteroptera: Lygaeidae). *Nytt Mag. Zool.*, **7**: 1–132.

LOHER, W., 1960. The chemical acceleration of the maturation process and its hormonal control in the male of the desert locust. *Proc. Roy. Soc. London, Ser. B*, **153**: 380–397.

NOVÁK, V. J. A., 1960. Insektenhormone (2nd Ed.), NČSAV, Prague.

PFLUGFELDER, O., 1958. Entwicklungsphysiologie der Insekten (2nd Ed.). Akademische Verlagsgesellschaft, Leipzig.

SÄGESSER, H., 1960. Über die Wirkung der Corpora allata auf den Sauerstoffverbrauch bei der Schabe *Leucophaea maderae* (F). *J. Ins. Physiol.*, **5**: 264–285.

SCHARRER, B., 1946. The relationship between corpora allata and reproductive organs in adult *Leucophaea maderae* (Orthoptera). *Endocrinology*, **38**: 46–55.

SCHARRER, B., AND M. VON HARNACK, 1958. Histophysiological studies on the corpus allatum of *Leucophaea maderae*. I. Normal life cycle in male and female adults. *Biol. Bull.*, **115**: 508–520.

SLÁMA, K., 1960. Oxygen consumption during the postembryonic development of *Pyrrhocoris apterus* and its comparison with that of Holometabola. *Ann. Ent. Soc. Amer.*, **53**: 606–610.

SLÁMA, K., 1964. Hormonal control of respiratory metabolism during growth, reproduction, and diapause in female adults of *Pyrrhocoris apterus* L. (Hemiptera). *J. Ins. Physiol.*, **10**: 283–303.

SLÁMA, K., AND H. HRUBEŠOVÁ, 1963. Übereinstimmung in der Einwirkung von larvalen und imaginalen Corpora allata auf den Respirationsmetabolismus und die Reproduktion bei *Pyrrhocoris apterus* L. Weibchen. *Zool. Jahrb. Physiol.*, **70**: 291–300.

THOMSEN, E., 1942. An experimental and anatomical study of the corpus allatum in the blowfly, *Calliphora erythrocephala* Meig. *Vidensk. Medd. naturh. Foren. Kbh.*, **106**: 320–405.

THOMSEN, E., 1949. Influence of the corpus allatum on the oxygen consumption of adult *Calliphora erythrocephala* Meig. *J. Exp. Biol.*, **26**: 137–149.

THOMSEN, E., AND I. MØLLER, 1963. Influence of neurosecretory cells and of corpus allatum on intestinal protease activity in the adult *Calliphora erythrocephala* Meig. *J. Exp. Biol.*, **40**: 301–321.

USHATINSKAYA, R. S., 1957. Osnovy kholodostoikosti nasekomykh. Izdatelstvo Akademii Nauk SSSR, Moscow.

WIGGLESWORTH, V. B., 1936. The function of the corpus allatum in the growth and reproduction of *Rhodnius prolixus* (Hemiptera). *Quart. J. Micr. Sci.*, **79**: 91–121.

WIGGLESWORTH, V. B., 1954. The Physiology of Insect Metamorphosis. Cambridge University Press, Cambridge.